Supporting information

A dimeric chlorite dismutase exhibits O_2-generating activity and acts as a chlorite antioxidant in *Klebsiella pneumoniae* MGH 78578

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Contents:

Figure S1.

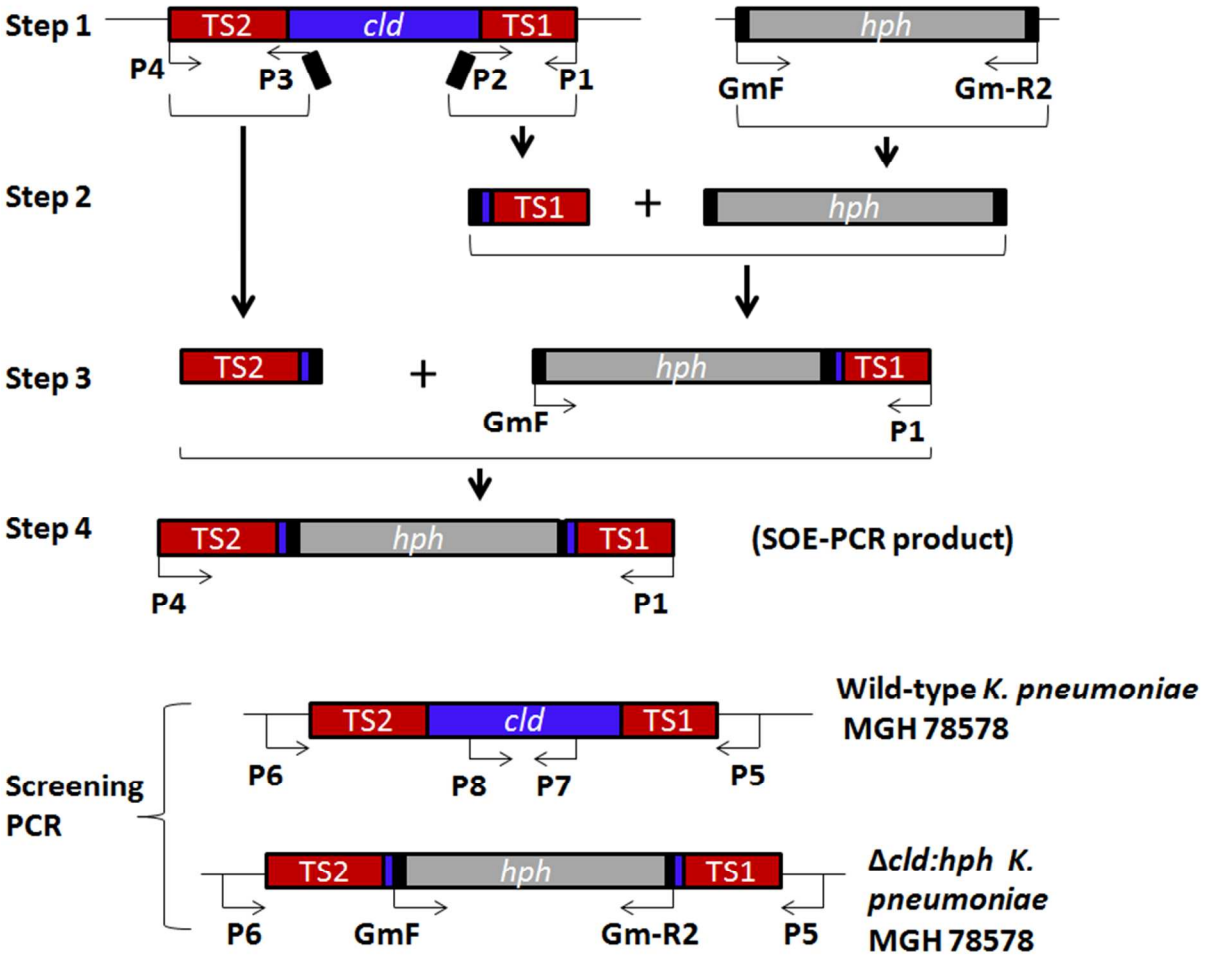


Figure S1. Schematic outlining the SOE-PCR and PCR validation strategy used to generate the Δ*cld* strain. See text for further details.

Figure S2.

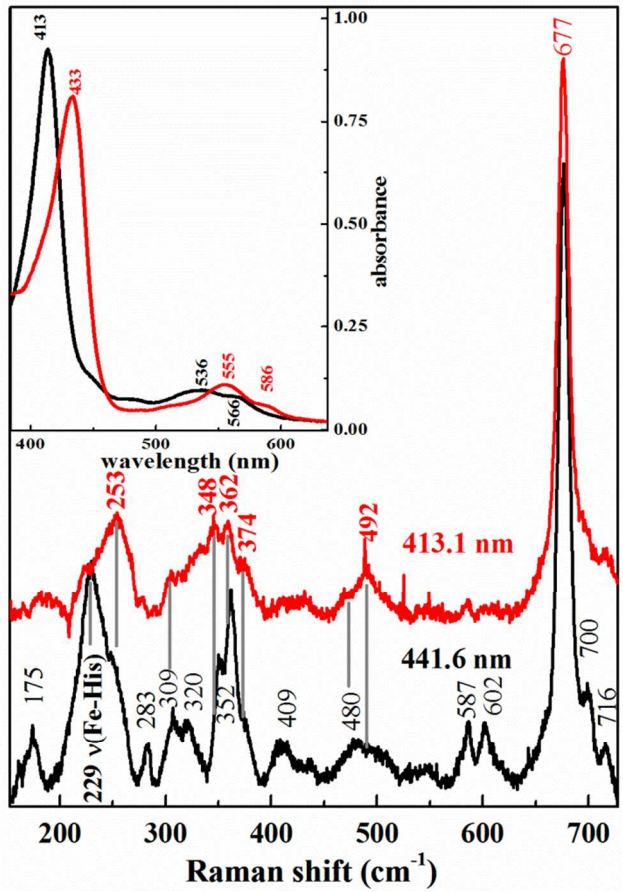


Figure S2. The iron histidine stretch of ferrous *Kp*Cld is resonance enhanced with 441.6 nm excitation. Comparison of rR spectra of ferrous *Kp*Cld pH 7.0 with 413.1 (red) and 441.6 nm (black) excitation. Inset: UV-visible spectra of ferric (black) and ferrous (red) KpCld pH 7.0 in 100 mM potassium phosphate buffer.

Figure S3.

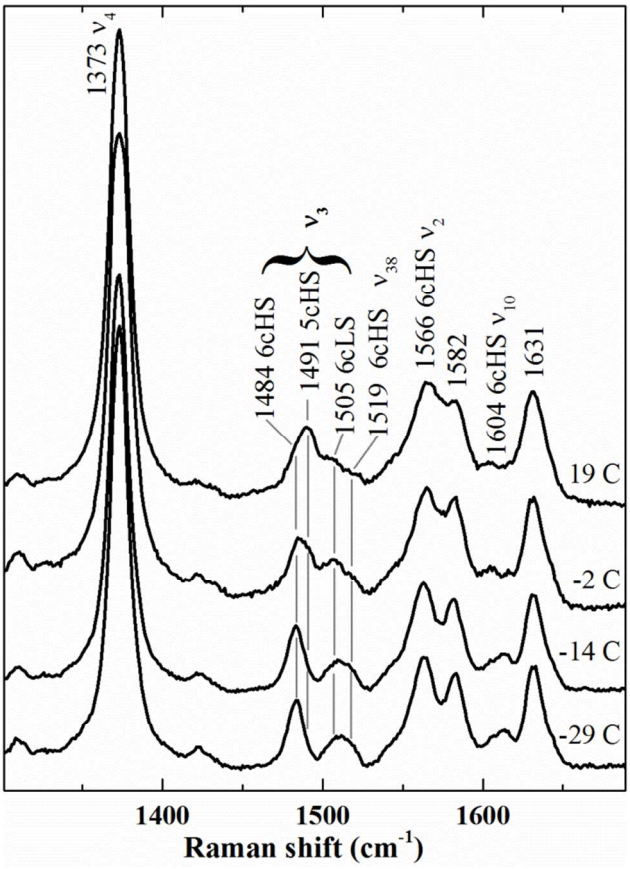


Figure S3. Temperature dependence of the rR spectrum of ferric *Kp*Cld pH 6.0 indicates exothermic binding of water to the heme. The high frequency rR spectra of ferric *Kp*Cld in 100 mM potassium phosphate pH 6.0 at the indicated temperatures were acquired with 413.1 nm excitation and 4 mW power at the sample. Temperature was controlled with a liquid N_2 boil off system. As the temperature is decreased, the broad ν_3 envelop encompassing contributions from 5cHS and 6cHS heme species sharpens to a symmetrical band consistent with a 6cHS aqua *Kp*Cld complex. As the 6cHS grows in, as judged by sharpening to the ν_3 band, ν_{38} associated with this 6cHS complex also becomes prominent and overlaps with the ν_3 that suggests a small amount of 6cLS species at room temperature. Band assignments were made by analogy to metMb.

Figure S4.

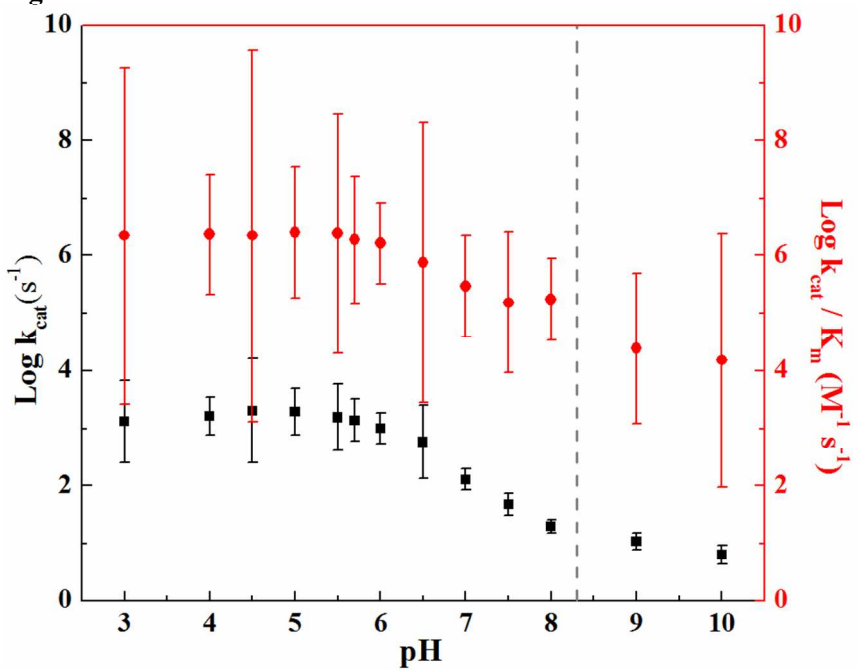


Figure S4. Plots of the log(k_{cat}) (black squares) and the log(k_{cat}/K_m) (red circles) for the chlorite decomposition reaction as a function of pH. Initial rates of chlorite-decomposing activity in the steady state were measured with a luminescence based probe used to measure O_2 evolution by *Kp*Cld. Samples were 12 nM WT *Kp*Cld and 0.05 – 2 mM chlorite in the following buffer solutions: 50 mM phosphate-citrate (pH < 6), 100 mM phosphate (pH 6-8), or glycine (pH >8). Plots of log(k_{cat}) and the log(k_{cat}/K_m) both show a single clear turning point corresponding to a pK_a of 7.0. This enzyme-localized (as opposed to heme-localized) deprotonation event decreases the enyzme activity and efficiency. The optimum activity is observed near pH 5.0 where k_{cat} and k_{cat}/K_M have values of 1.9 (±0.2) × 10^3 s^{-1} and 2.5 ± (0.4) x 10^6 $M^{-1}s^{-1}$, respectively. Above the enzyme-localized pK_a , k_{cat} and k_{cat}/K_M drop to 1.1 (±1.6) × 10^1 s^{-1} and 2.4 ± (0.7) x 10^4 $M^{-1}s^{-1}$(pH 9.0), respectively.

Figure S5.

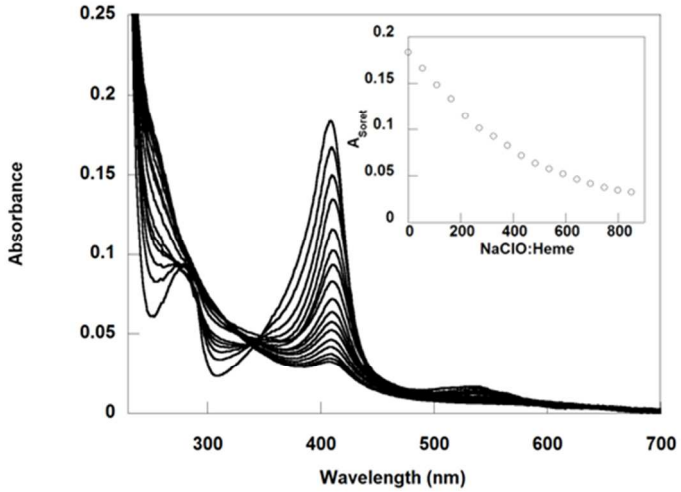


Figure S5. Titration of *Kp*Cld heme spectrum with NaClO. 50µL of 7.3 µM heme-bound KpCld was added to 950µL citrate-phosphate buffer (50 mM, pH 6.0, room temperature) in a UV/vis cuvette. A defined concentration of sodium hypochlorite was added and the solution was rapidly mixed. (Shown here is 0.90 mM an amount sufficient to nearly eliminate the heme Soret band. Several concentrations of NaClO were examined.) Spectra were scanned every 6s until the Soret band no longer changed. The inset shows the final Soret band absorbance plotted versus increasing [NaClO], generated from a series of experiments like the representative one shown here. The curve reaches an asymptote near 800 equivalents of NaClO per heme.

Figure S6.

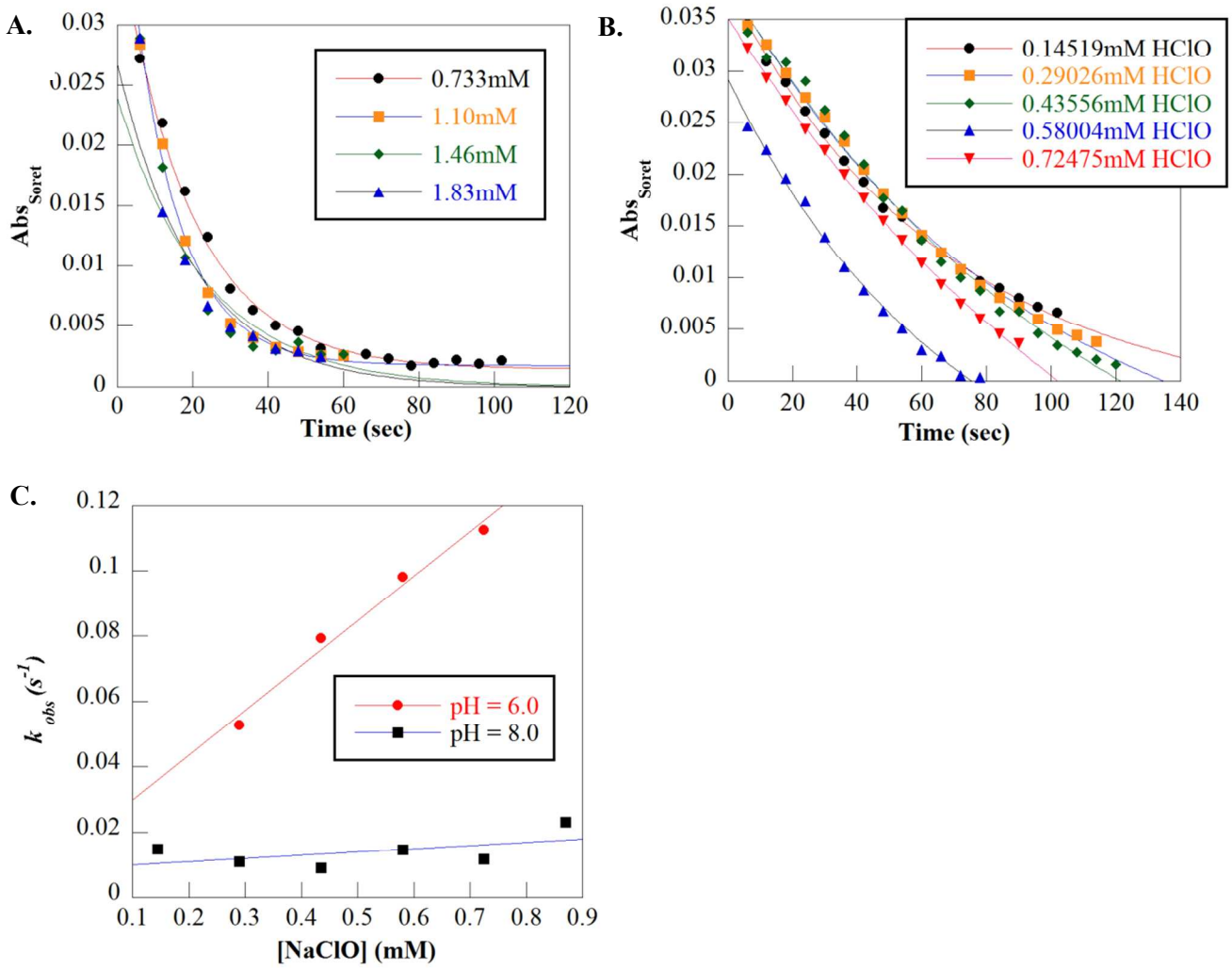
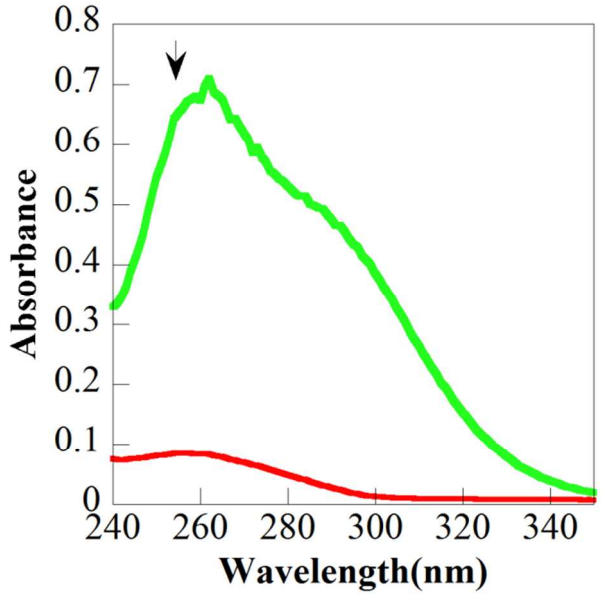


Figure S6. 50µL of 7.3 µM heme-bound KpCld was added to 950 µL 50 mM citrate-phosphate buffer, pH 6.0 or 8.0, in a UV/vis cuvette at room temperature. NaClO was added to final concentrations ranging from 0.15mM – 0.9mM. Immediately following each NaClO addition, the solution was mixed rapidly by pipetting up and the reaction monitored over time in scanning mode (250-700 nm) with scans every 6s. The reaction was monitored to completion, until the Soret band was eliminated and/or changes in the spectra ceased. First order rate constants (k_{obs}) were fit to the resulting single exponential curves (A and B, pH 6 and 8 respectively) describing the change in the Soret band absorbance versus time. The values of k_{obs} were plotted versus [NaClO] to generate a second-order rate constant from the slope of the line (C).

Figure S7.

A.



B.

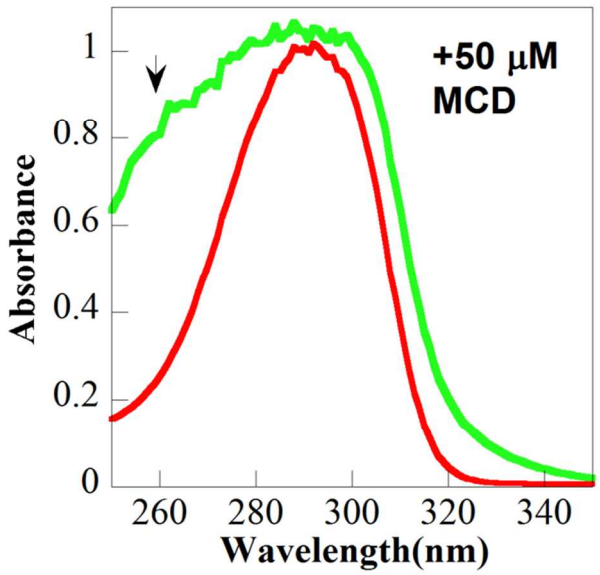


Figure S7. Trappable HOCl is not formed in appreciable quantities during the *Da*Cld reaction. The reaction between *Da*Cld and chlorite was monitored under conditions similar to those shown in the text in Figure 5A-C. Only the initial spectra (t_0, green) and final spectra (t_{30min}, red) are shown. **A.** 5mM ClO_2^- was added to 0.1µM of *Da*Cld in 50 mM potassium phosphate buffer, pH 7.4. 88% of the initially present ClO_2^- was degraded, according to iodometric titration of the end products. B. The reaction was run under the same condtions but in the presence of 50 mM MCD (λmax ~ 290 nm). While most of the chlorite(λmax ~ 260 nm) is eliminated under these conditions, little MCD is converted to its chlorinated product DCD, which has no absorbance at this wavelength.

Table S1. Bacterial strains and plasmids used in this study

Strain/ plasmid	Genotype/Features	Reference /Sources
Strain		
***Klebsiella pneumoniae* MGH 78578**	Wild-type *K. pneumoniae* strain	ATCC
KR3478	*K. pneumoniae* MGH 78578 carrying pKOBEGApra	This study
KR3444	Δ*cld K. pneumoniae* MGH 78578 (Colony A)	This study
KR3523	Δ*cld K. pneumoniae* MGH 78578 (Colony B)	This study
Plasmid		
pKOBEGApra	Arabinose-inducible lambda Red expression plasmid; apramycin resistance	1
pJTAG-hyg	FRT-*hph*-FRT, hygromycin resistance cassette	2

1. **Chaveroche MK, Ghigo JM, d'Enfert C**. 2000. A rapid method for efficient gene replacement in the filamentous fungus *Aspergillus nidulans*. Nucleic Acids Res 28: E97.
2. **Zhang Y, Jiang X, Wang Y, Li G, Tian Y, Liu H, Ai F, Ma Y, Wang B, Ruan F, Rajakumar K**. 2014. Contribution of β-Lactamases and porin proteins OmpK35 and OmpK36 to carbapenem resistance in clinical isolates of KPC-2-producing *Klebsiella pneumoniae.* Antimicrob Agents Chemother 58:1214–1217.

Table S2. Oligonucleotide primers used in this study.

Primer number	Primer Name	Use/ target	Sequence (5'→3')[a]
-	GmF	FRT-*hph*-FRT	CGAATTAGCTTCAAAAGCGCTCTGA
-	GmR	FRT-*hph*-FRT	CGAATTGGGGATCTTGAAGTTCCT
-	EBGNHe-5	*gam, bet, exo*	CCCGCTAGCGAAAAGATGTTTCGTGAAGC
-	EBGh3-3	*gam, bet, exo*	GGGAAGCTTATTATCGTGAGGATGCGTCA
P1	cld-TS1-R	TS1	CCGTTATGTCATGCCTACCC
P2	cld-TS1-Fp/GmF	TS1	TCAGAGCGCTTTTGAAGCTAATTCGATTCATCAGTTTCCTCTCAG
P3	cld-TS2-Rp/GmR	TS2	AGGAACTTCAAGATCCCCAATTCGTAGTATCAGGTTTAACTGCG
P4	cld-TS2-F	TS2	CAACAACTGGGTGGAAAACC
P5	cld-upstr-TS1-Rp	Screening	CAATTGGAACGGGGGCTTTG
P6	cld-downstr-TS2-Fp	Screening	GAACCTTCCTGGGTGACTGG
P7	cld-internal-F	*cld*	AAGTCGAAGGGTTCGCTCTC
P8	cld-internal-R	*cld*	TCGCGGGATAACGAGTAACG

[a] Underlined nucleotides corresponding to primers P2 and P3 are complementary to matching sequences of primers GmF and GmR, respectively.